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New pharmacological strategies for treatment of Alzheimer's disease: focus on disease modifying drugs

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Current approved drug treatments for Alzheimer disease (AD) include cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and the NMDA receptor antagonist memantine. These drugs provide symptomatic relief but poorly affect the progression of the disease. Drug discovery has been directed, in the last 10 years, to develop 'disease modifying drugs' hopefully able to counteract the progression of AD. Because in a chronic, slow progressing pathological process, such as AD, an early start of treatment enhances the chance of success, it is crucial to have biomarkers for early detection of AD-related brain dysfunction, usable before clinical onset. Reliable early biomarkers need therefore to be prospectively tested for predictive accuracy, with specific cut off values validated in clinical practice. Disease modifying drugs developed so far include drugs to reduce β amyloid (Aβ) production, drugs to prevent Aβ aggregation, drugs to promote Aβ clearance, drugs targeting tau phosphorylation and assembly and other approaches. Unfortunately none of these drugs has demonstrated efficacy in phase 3 studies. The failure of clinical trials with disease modifying drugs raises a number of questions, spanning from methodological flaws to fundamental understanding of AD pathophysiology and biology. Recently, new diagnostic criteria applicable to presymptomatic stages of AD have been published. These new criteria may impact on drug development, such that future trials on disease modifying drugs will include populations susceptible to AD, before clinical onset. Specific problems with completed trials and hopes with ongoing trials are discussed in this review.

Introduction

Alzheimer's disease (AD) is a common disorder characterized by cognitive decline [1] associated with the presence of β-amyloid (Aβ) in plaques, intracellular aggregates of tau protein, forming neurofibrillary tangles (NFT) and progressive neuronal loss [2]. Aβ plays a primary role in AD pathophysiology [2]. Oligomer species of aggregated Aβ exert toxic effects on synaptic and cellular functions, finally leading to neurodegeneration and cognitive, as well as neuropsychiatric, symptoms [3]. Current treatment of AD includes cholinesterase inhibitors (donepezil, rivastigmine, galantamine), used for mild to moderate AD, and the NMDA receptor antagonist, memantine, approved for the treatment of moderate to severe AD [4, 5]. These drugs mainly provide symptomatic, short-term benefits, without affecting the underlying pathogenic mechanisms of the disease [4], though a neuroprotective potential has also been proposed [6, 7]. Developing disease modifying drugs, able to counteract the progression of AD, is one of the biggest challenges of modern pharmacology. The pathophysiological process of AD begins many years before clinical diagnosis is set. The optimal time for disease-modifying drug treatment may therefore be in the presymptomatic stage of AD, where the disease is still hidden. Recently, the criteria for the clinical diagnosis of AD have been revised by the National Institute on Ageing and the Alzheimer's Association workgroup [8]. The new criteria incorporate biomarkers to identify early stages of AD, susceptible to being treated with disease modifying drugs [9, 10].

In the present review, we will summarize the new pharmacological strategies for the treatment of AD, focusing

Table 1

Current status of clinical development of some disease modifying drugs for treatment of Alzheimer's disease (AD)

Drug	Mechanism of action relevant for AD	Phase of study	Result of study	Caveat of study
Rosiglitazone	β-secretase inhibition (?)	3	Ineffective	Lack of biomarker
Semagacestat	γ-secretase inhibition	3	Premature end	Severe adverse drug reaction
Tarenflurbil	γ-secretase modulation	3	Ineffective	Low potency, blood–brain barrier passage
Tramiprosate	Inhibition of Aβ oligomerization	3	Ineffective	–
Scyllo-inositol	Inhibition of Aβ oligomerization	2	Ineffective	Biomarker change
Bapineuzumab	Aβ clearance	3	Ongoing	Vasogenic oedema, amyloid angiopathy
Solaneuzumab	Aβ clearance	3	Ongoing	–
Lithium	Inhibition of tau phosphorylation	2	Clinical improvement Decrease of P-tau in CSF	–
Methylthioninium chloride	Inhibition of tau aggregation	2	Clinical improvement with 60 mg day^{-1}	Lack of biomarker
Nilvadipine	Aβ clearance	Open label	Clinical improvement	Lack of biomarker
Latrepirdine	Mitochondrial protection	3	Ineffective	–
		3	Ongoing (in association with other drugs)	–

our attention on potential disease modifying drugs currently studied in phase 3 clinical trials. A summary of the current status of the clinical development of some disease modifying drugs is shown in Table 1.

Disease modifying drugs: definition and implications for drug development in AD

A disease modifying drug is an agent that slows the progression of structural damage, such that its effect is persistent and can be detected even after stopping the treatment, because the cumulative pathological changes would be less severe in the treated group as compared with the control (placebo) group. In contrast, the definition 'symptomatic drug' refers to an agent that does not alter the progression of the disease, but only decreases (palliates) the severity of symptoms. The symptomatic effect is usually reversible, such that, if the treatment is interrupted, the treated group might be indistinguishable from the control (placebo) group. Definition and validation of appropriate biomarkers and scales of clinical outcome are of paramount importance for assessing efficacy of disease modifying drug treatments for AD. Agents that target the underlying pathophysiology of AD are expected to have greater effect on biomarker levels and disease progression before any substantial, irreversible functional loss occurs [11]. Biological markers of AD may be divided into different classes according to the 'amyloid' hypothesis. Biomarkers of brain Aβ amyloidosis include both reduction in $A\beta_{42}$ in cerebrospinal fluid (CSF) [12] and positron-emission tomography (PET) evidence of Aβ deposition, using a variety of specific ligands [13]. Elevated tau in CSF seems related to neuronal injury, but is not specific for AD. However, the association of elevated tau with low concentrations of $A\beta_{42}$ in CSF is considered the most informative biomarker of AD. Furthermore, low $A\beta_{42}$ in CSF together with elevated tau might help in predicting the progression of patients with mild cognitive impairment (MCI) to AD [9]. In this respect, a recent report shows, in a presymptomatic carrier of an APP mutation, decrease of $A\beta_{42}$ and increase tau concentrations in CSF, with substantial changes in a 5 year, symptom free, interval [14]. Further studies are needed, both in early onset AD and late onset AD patients, to confirm whether these CSF biomarkers might be sensitive indicators of presymptomatic disease.

Other biomarkers, such as PET measurement of fluorodeoxyglucose 18F (FDG) uptake and magnetic resonance imaging (MRI) of brain atrophy, track indices of synaptic dysfunction and neuronal injury and are less specific [12]. However, all together these biomarkers may be very helpful in the early detection of AD-related brain dysfunction. In fact, studies conducted in carriers of AD genetic risk factors, have demonstrated the presence of Aβ accumulation in CSF, positive PET amyloid imaging, FDG-PET hypometabolism and functional MRI abnormalities up to a decade before the clinical onset of AD [10, 12]. These biomarkers need to be prospectively tested for predictive accuracy. Moreover, specific cutoff values need further validation in clinical practice. Neuropsychological and neurobehavioural tools to detect the earliest clinical manifestations of AD might be particularly useful in monitoring the response to disease modifying therapies in amnestic MCI patients, that have a prominent impairment in episodic memory and positive biomarkers [9]. Because AD is slowly progressing, demonstrating the effectiveness of a disease modifying treatment might require years. Most clinical studies examine 18–24 months of active treatment compared with placebo, but should provide informative data for a much longer period of time, given that patients are likely to take these medications for many years in clinical practice.

Up to now no disease modifying drugs are available for AD. Several have been tested, down to phase 3, but none

has yet reached approval. The failure of clinical trials with disease modifying drugs raises a number of questions, spanning from methodological flaws to fundamental understanding of AD pathophysiology and biology. Some problems may arise from publication bias that favours positive results [15, 16], biomarkers and clinical outcomes utilized in animal models that substantially differ from human studies and time course of treatment in relation to development of disease, i.e. clinical studies enrol symptomatic patients, where some degree of neurodegeneration is already in place. Since the original Alzheimer's description [17], Aβ production and deposition has been considered as the main activity responsible for the pathological mechanism of AD, because it was documented in amyloid plaques of AD subjects by *post mortem* analysis. This view is referred to as the 'Aβ hypothesis'. The Aβ hypothesis has recently been challenged by the observation that Aβ clearing is not necessarily accompanied by cognitive improvement [2, 18, 19]. The physiological role of Aβ peptides, encoded also in the genome of the normal (healthy) population, has just begun to be unravelled and might be involved in basic mechanisms of cognition and memory, such as long-term potentiation (LTP) [20]. Proper folding and aggregation state of Aβ, rather than its absolute concentration, seems to be the determinant of neuronal toxicity in AD [21]. While assessing Aβ folding and aggregation state *in vitro* or *post mortem* in brain tissues is achievable [22, 23], this is not feasible, at present, in the living human brain, which makes the use of parenchymal Aβ as an AD biomarker very difficult.

Drugs to reduce Aβ production

As shown in Figure 1, generation of $A\beta_{40}$ or $A\beta_{42}$ is the result of two sequential cleavages of the amyloid precursor protein (APP). First, extracellular cleavage of APP by β-secretase 1 (also termed beta-site amyloid precursor protein cleaving enzyme 1 or BACE1) produces a soluble extracellular fragment and a cell membrane-bound fragment referred to as C99. Subsequent cleavage of C99 within its transmembrane domain by γ-secretase releases the intracellular domain of APP and generates Aβ (Figure 1). In contrast, initial cleavage of APP by α-secretase prevents generation of Aβ, because, by cleaving APP closer to the cell membrane than β-secretase does, it removes a fragment of Aβ (Figure 1) [24]. Therapeutic attempts have targeted inhibition of β-secretase and γ-secretase.

β-secretase 1 is an aspartyl protease that shares some features with HIV aspartyl proteases [25]. No known mutations in the gene encoding β-secretase have been related to familial AD, but elevated levels of this enzyme have been found in sporadic AD [26] and might be associated with polymorphism in the promoter region [27]. Because β-secretase 1 also has other substrates (including neuregulin-1, which is involved in myelination), development of inhibitors may theoretically face problems of toxicity related to non-specific effects, though deletion of the β-secretase 1 gene produces only minor phenotype changes [28]. The thiazolidinediones, rosiglitazone and pioglitazone, that have been tested for AD in randomized controlled trials (RCTs), may in part act as suppressors of β-secretase expression [29]. Chang *et al*. reported recently [30] that the administration of a β-secretase inhibitor rescued cognitive decline and reduced brain Aβ in AD mice Tg2576, with no toxicity over a 7 month time period. Up to now no efficacy data are available from phase 3 clinical trials of β-secretase inhibitors. Specific problems in developing safe, non-toxic β-secretase inhibitors are related to blood–brain barrier (BBB) penetration and reasonable selectivity. Some interesting compounds have been designed by using crystal structure based inhibitor design [31] and some have been tested or currently are in phase 1 trials [32]. As mentioned above, rosiglitazone is an antidiabetic drug that has been clinically tested in AD. The main mechanism of action of rosiglitazone in diabetes, i.e. PPARγ binding and subsequent transcription of genes involved in metabolic control, is precisely defined at the molecular level [33]. The same, however, cannot be stated for a supposed beneficial effect of rosiglitazone in AD. Starting from a correlation between insulin resistance and AD [34], preclinical studies looked for an effect of rosiglitazone in animal models of AD, without, however, a precisely defined testable hypothesis in terms of molecular and cellular mechanisms [35]. Rosiglitazone was shown to improve spatial learning and memory abilities, slightly decrease $A\beta_{42}$ concentrations in brain (but not $A\beta_{40}$) and induce insulin-degrading enzyme (IDE), without affecting the amyloid plaque burden in Tg2576 mice [36]. IDE is a thiol metalloprotease that degrades insulin as well as monomeric Aβ [37], whose expression has been shown to be PPARγ-dependent in neurons [38]. However, the quantitative contribution that IDE may give to Aβ turnover in brain parenchyma remains to be determined, and PPARδ, rather than PPARγ, may have a stronger effect in expression of Aβ degrading enzymes [39]. In one phase 2 study, after 6 months treatment with rosiglitazone, patients with mild AD or amnestic MCI exhibited better delayed recall and selective attention as compared with the placebo group [40]. The only biomarker used was Aβ in plasma, which was decreased in the placebo group [40]. This finding was interpreted by the authors as an index of Aβ deposition in brain, potentially contributing to clinical worsening, an explanation that cannot be considered satisfactory, because, at variance with Aβ in CSF [13, 41], circulating Aβ does not provide reproducible correlation with AD [42] and does not reflect Aβ processing in the brain [43]. In another phase 2 study, mild to moderate AD patients were treated with three different doses of rosiglitazone for 24 weeks and the data were stratified according to the APOE ε4 allele status. In APOE ε4 non-carriers rosiglitazone seemed to determine a cognitive and functional improvement, whereas APOE ε4

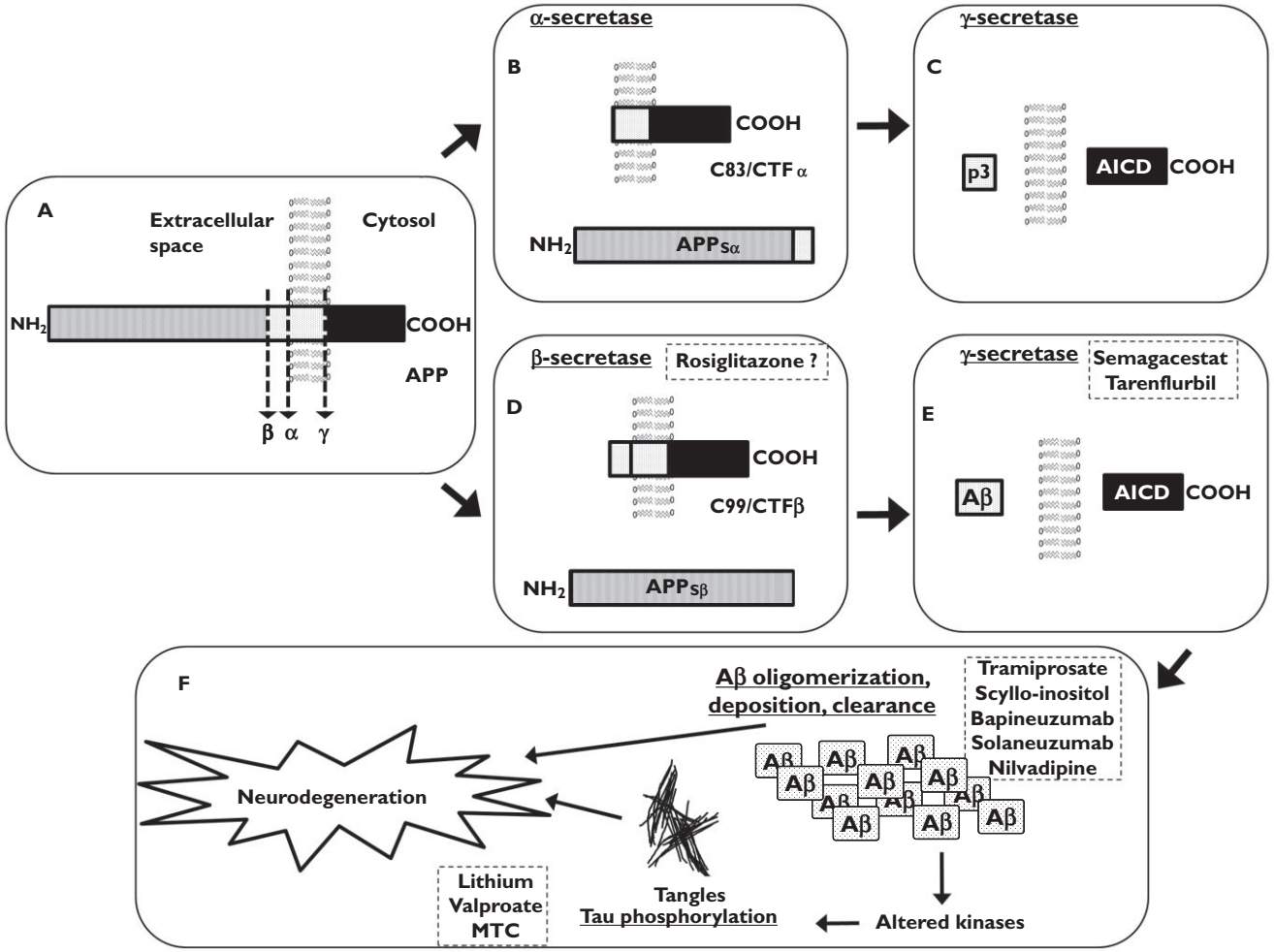

Figure 1

Main steps of sequential cleavage of amyloid precursor protein (APP), leading to generation of β amyloid (Aβ) and/or other products. In A, dashed arrows indicate the cleavage sites for α-, β- and γ-secretase. In B, ectodomain shedding of APP by α-secretase gives a soluble extracellular APP fragment (APPsα) and a 83 amino acid, membrane-bound, carboxy terminus fragment (C83/CTFα). Subsequent intramembrane proteolytic cleavage of C83/CTFα by γ-secretase (in C) releases a short extracellular p3-peptide (p3) and a cytosolic APP intracellular domain (AICD). In D, ectodomain shedding of APP by β-secretase gives a soluble extracellular APP fragment (APPsβ) and a 99 amino acid, membrane-bound, carboxy terminus fragment (C99/CTFβ). Subsequent intramembrane proteolytic cleavage of C99/CTFβ by γ-secretase (in E) releases extracellular Aβ and a cytosolic APP intracellular domain (AICD). In F, Aβ oligomerization and deposition lead to neurodegeneration, both directly and through tau hyperphosphorylation. In dashed boxes, disease modifying drugs that interfere with each particular step. See text for more information and references

allele carriers showed no beneficial effect [44]. Of note, no specific AD biomarker was assessed in this study. Analysis of data according to the APOE ε4 allele status was based on the hypothesis that ε4-positive individuals, susceptible to early onset AD [45], have disturbances in enzymatic pathways of glucose metabolism in the brain, reminiscent of insulin resistance as seen in type 2 diabetes [46, 47], that might be sensitive to PPARγ activation by rosiglitazone. In contrast with the initial hypothesis, however, a mild clinical benefit was seen in ε4 non-carriers. The subsequent larger phase 3 study showed no significant clinical benefit of rosiglitazone in whatever APOE genetic population examined [48], not confirming the preliminary observation made in the phase 2 studies. The overall criticism that might be formulated (retrospectively) here is that two elements that may increase the chance of success in a clinical study, a precise biological hypothesis and a quantitative assessable biomarker, were lacking.

γ-secretase is a protease complex that cleaves proteins at residues within their single membrane spanning domain. The most known substrate of γ-secretase is APP, whose cleavage produces Aβ. The γ-secretase complex consists of four individual proteins, presenilin, nicastrin, APH-1 and PEN-2 [49]. A fifth protein, known as CD147, acts as a negative regulator of the complex [50]. Presenilin is the catalytic subunit and mutations in the presenilin gene rep-

resent a major genetic risk factor for AD [51]. Although γ-secretase mutations that completely knock out enzyme function prevent generation of Aβ, mutations that only partially knock out enzyme function often enhance generation of Aβ, a finding ascribed to a gain of function [52]. Hence, γ-secretase inhibitors may enhance the production of $Aβ_{42}$ while blocking other γ-secretase activities, thus mimicking the effects of PS mutations [52], which may, at least in theory, produce paradoxical outcomes in AD trials (increased Aβ deposition and cognitive worsening). Furthermore, development of γ-secretase inhibitors as disease modifying drugs presents problems related to potential non-specific effects. This is because γ-secretase is not only responsible for Aβ generation but is also involved in intramembranous cleavage of several proteins, including the Notch receptor, ErbB4, p75NTR neurotrophin receptor, N-cadherin and the sodium channel β4 subunit [53]. Semagacestat was the first γ-secretase inhibitor to undergo extensive clinical testing and was shown to reduce Aβ concentrations in plasma and Aβ production in the central nervous system (CNS) [54, 55]. Two large phase 3 RCTs with semagacestat were prematurely stopped because of some serious collateral adverse effects, including haematological, gastrointestinal and skin toxicity, that have been attributed to inhibition of the Notch signaling pathway [56]. Furthermore, in these studies, no improvement or moderate worsening of cognition was observed, perhaps related to γ-secretase inhibition within the CNS [57–59]. Notch-sparing γ-secretase inhibitors (second generation inhibitors) and/or modulators (agents that shift γ-secretase cleavage activity from longer to shorter β-amyloid species, without affecting Notch cleavage) are in clinical development. Begacestat [60], BMS-708163 [61], PF-3084014 [62] and CHF-5074 [63] display a 10–100 fold selectivity on APP over the Notch cleavage. Some non-steroidal anti-inflammatory drugs (NSAIDs) act as γ-secretase modulators, decreasing $Aβ_{40}$ and $Aβ_{42}$, while increasing $Aβ_{38}$ [53]. Tarenflurbil (the R-enantiomer of flurbiprofen) was tested in phase 3 RCTs but did not appear to slow cognitive decline [19], while increasing frequency of dizziness, anaemia, and infection [19]. The failure of tarenflurbil may be ascribed to low potency and poor brain penetration [64]. Furthermore, cyclo-oxygenase inhibition in microglia may result in inhibition of Aβ clearance [65].

Other drugs, such as 1,4-dihydropyridine (DHP) L-type calcium channel blockers, are known to interfere with Aβ production. Different large population-based studies have demonstrated that certain DHP calcium channel blockers used for the treatment of hypertension, such as nilvadipine, can reduce the risk of developing AD [66, 67]. Recent studies suggest that such benefits are not related to the drug's blood pressure lowering function [68]. Both nilvadipine and amlodipine decrease Aβ production from APP *in vitro*, but only chronic oral treatment with nilvadipine reduces Aβ accumulation in a transgenic model of AD, by targeting both production and clearance of Aβ across the BBB [68]. In a small study, nilvadipine slowed cognitive decline in MCI patients with hypertension [69]. Nilvadipine stabilizes cognition [70] and is well tolerated, with no dangerous blood pressure lowering effects [70]. A multicentre phase 3 clinical trial will start in January 2012 to assess the efficacy of nilvadipine as a disease modifying drug in AD patients (http://www.alzforum.org/new/detail.asp?id=2838).

Drugs to prevent Aβ aggregation

Aggregation of monomeric Aβ species into higher molecular weight oligomers produces the primary neurotoxic species in AD [71, 72]. Tramiprosate (3-amino-L-propanesulfonic acid) is a glycosaminoglycan that binds to Aβ monomers and prevents formation of oligomers, thus enhancing Aβ clearance from the brain [73]. An initial, phase 2 study showed that tramiprosate reduces $Aβ_{42}$ concentrations in CSF [74]. In a larger, phase 3 study, however, tramiprosate did not determine clinical improvement [75], although a recent subanalysis suggests that it may exert some beneficial effects on memory, language and praxis skills [76], requiring further clinical evaluation.

Because zinc and copper are catalysts for Aβ aggregation and stabilization of amyloid plaques, chelating agents may be effective in dissolving amyloid deposits *in vitro* and *in vivo*. PBT2 is an 8-hydroxy quinolone, orally administered and with good BBB permeability, that removes copper and zinc from CSF, promotes Aβ oligomer clearance and restores cognition in AD mouse models [77, 78]. In a recent phase 2a study, PBT2 lowered $Aβ_{42}$ in CSF and improved cognition, but no correlation was found between Aβ in CSF and cognitive changes [77].

Scyllo-inositol (scyllo-cyclohexanehexol, AZD-103, ELND-005) is an orally administered stereoisomer of inositol that crosses the BBB using inositol transporters. Scyllo-inositol can directly bind to Aβ oligomers promoting dissociation of Aβ aggregates [79, 80]. Interestingly, TgCRND8 mice treated with AZD-103 show a 25% reduction of Aβ oligomers with a concomitant increase in monomeric species (+133%), suggesting that this drug can prevent the transition from Aβ monomers to Aβ oligomers [80]. Recently, a phase 2 clinical trial (NCT00568776) evaluating safety, efficacy and effects on biomarkers of ELND-005 in mild to moderate AD patients has been completed [81]. Of the three tested doses, 250, 1000 and 2000 mg, only 250 was well tolerated, whereas side effects in the two higher dose groups led to early discontinuation. In spite of lack of significant clinical improvement, patients receiving 250 mg of ELND005 had an increase in their brain ventricular volume as well as a reduction in CSF $Aβ_{42}$. Large-scale phase 3 clinical studies are needed to evaluate the clinical efficacy of ELND005.

Additional small molecules, including polyphenolic compounds such as curcumin (–)-epigallocatechin-3-

gallate (EGCG) and grape seed extract, attenuate Aβ aggregation [80, 82]. EGCG has shown good tolerability (NCT00525668) and is currently being evaluated in a phase 2–3 RCT (NCT00951834).

Drugs to promote Aβ clearance

Immunotherapy toward Aβ is considered one of the most promising approaches to develop disease modifying drugs in AD, because it can potentially affect production, aggregation and deposition of Aβ [83, 84]. Active immunization by vaccination promotes formation of antibodies against pathogenic forms of Aβ, by stimulating an immune response, whereas passive immunotherapy supplies antibodies from an exogenous source [83]. Active Aβ immunotherapy has been studied and validated since 1999, when it was demonstrated that generation of Aβ antibodies resulted in clearance of cerebral Aβ by microglial phagocytosis of antibody-opsonized Aβ deposits [85]. Aβ immunotherapy improves cognitive deficits in AD models and lowers plaque load in non-human primates. Unfortunately, a phase 2 clinical trial of active immunization using full length human $A\beta_{42}$ peptide with QS-21 adjuvant was stopped prematurely because some patients developed brain inflammation with aseptic meningoencephalitis [86]. T cell recognition of the human full length Aβ peptide may have induced an adverse autoimmune response [87]. Furthermore, although Aβ-specific antibodies clear brain amyloid plaques, they do not halt progressive neurodegeneration [88, 89] or affect vascular amyloid and hyperphosphorylated tau deposits [90]. Recent alternative approaches are based on shorter Aβ immunogens that target the N-terminus (strong B cell epitope) without affecting the mid-region and C-terminus (T cell epitopes) [84]. Because of the low responsiveness and adverse reactions to vaccines, passive immunotherapy has been proposed as an alternative strategy [91–93]. Passive immunotherapy, however, may also be associated with adverse effects such as vasogenic oedema and cerebral amyloid angiopathy with microhaemorrhages [5, 94, 95]. The most studied and advanced Aβ targeted antibody is bapineuzumab [96]. The efficacy and safety of bapineuzumab seem to be related to APOE allele status. In APOE ε4 carriers this drug can favour the onset of vasogenic oedema [93] that may limit its clinical use and has led to the abandonment of the highest dose of the drug (2 mg kg^{-1}) [97]. Lower doses of bapineuzumab are currently used in phase 3 trials in ε4 carriers, whereas slightly higher doses can be used in non- ε4 carriers [97]. To date seven phase 3 studies with bapineuzumab are ongoing (NCT00996918, NCT00574132, NCT00676143, NCT00667816, NCT00575055, NCT00998764 and NCT00937352). Another humanized anti-Aβ monoclonal antibody in advanced clinical development is solanezumab; three phase 3 trials are ongoing (NCT 01127633, NCT 00904683 and NCT00905372). Others antibodies in phase 1 and 2 trials include PF-04360365, GSK-933776, R-1415 and MABT-5102A.

Intravenous immunoglobulins (IVIG) contain naturally occurring autoantibodies that specifically recognize Aβ and block is toxic effects [98–101]. A phase 3 study with IVIG 10% is ongoing. Adekar *et al*. [102] showed that free human Igγ heavy chains (HC) possess anti-amyloidogenic activity because they bind to an amyloid, fibril-related, conformational epitope while not affecting native Aβ monomers. Free human Igγ HC offer the advantage of crossing the BBB and being less prone to adverse inflammatory side effects [103]. New strategies in the immunotherapy of AD should be directed to Aβ dimers and/or other toxic oligomers, preserving Aβ monomers, that may be involved in maintaining learning memory and neuronal survival [104]. Conformation specific antibodies, binding toxic Aβ oligomers without affecting Aβ monomers, have been recently developed [105].

Strategies targeting tau

NFTs are intracellular aggregates of paired helical filaments whose main constituent is a hyperphosphorylated form of the protein tau [106]. Expression pattern of NFTs correlates with the clinical onset and progression of AD [107]. Although Aβ and tau have been considered for years as distinct with regard to their role in AD pathogenesis, recent evidence suggests that these two proteins significantly interact and that tau-related events are essential for AD pathogenesis [108]. Findings obtained in a triple transgenic mouse model of AD [109, 110], suggest that the two major histopathological hallmarks of AD, i.e. Aβ deposits and NFT, containing hyperphosphorylated tau, lie along the same pathological cascade. Aβ accumulation precedes and drives tau hyperphosphorylation via the activation of different kinases such as cyclin dependent kinase 5 (CDK5) and glycogen synthase kinase 3β (GSK3β) [108, 109, 111, 112]. Tau hyperphosphorylation leads to destabilization of neuronal microtubular dynamics, which finally results in an impairment of synaptic function [106]. The critical role of tau in mediating Aβ-induced neurodegeneration has been demonstrated both in *in vitro* and *in vivo* models [113, 114]. Tau hyperphosphorylation and subsequent accumulation in the dendritic compartment increases the vulnerability of neurons to the toxic effects of Aβ [108, 115]. Recent efforts in drug discovery have been therefore directed to develop inhibitors of tau-phosphorylation and compounds that prevent tau aggregation and/or promote disassembly. GSK3β is the main enzyme involved in tau hyperphosphorylation [116]. Lithium and valproate, currently used as mood stabilizers, both inhibit GSK3β and reduce tau phosphorylation in animal models [117]. Valproate has been studied in the Alzheimer's Disease Cooperative Study (ADCS) [118]. In this study valproate did not

modify cognition and functional status but reduced agitation and psychosis [118]. A more recent meta-analysis, however, shows that valproate is ineffective against agitation in demented patients, and is also associated with an unacceptable rate of adverse effects, such as falls, infection and gastrointestinal disorders [119]. Lithium is neuroprotective in animal models of AD, not only via the inhibition of GSK-3β, but also through other mechanisms, including reduction of Aβ production [120, 121] and release of TGF-β1 [122]. In patients treated with lithium for psychiatric disorders, the risk of developing AD is reduced [123, 124]. Some studies in AD patients, however, have failed to demonstrate a positive effect of lithium on cognitive performance [5, 125, 126]. A recent single centre study showed that lithium reduced both cognitive decline and CSF concentration of P-tau in patients with amnestic MCI [127]. Safety problems related to lithium treatment in elderly people need specific attention and may lead to high discontinuation rates in AD patients [128]. Other inhibitors of GSK-3β have shown neuroprotective effects in preclinical models of AD [129]. A phase 2 RCT has been recently completed with NP031112 (NCT00948259).

Methylthioninium chloride (MTC), also known as methylene blue, is a promising compound which possesses antioxidative properties, reduces Aβ oligomerization and, most importantly, binds to the domain responsible for tau aggregation [130]. A phase 2b RCT study of MTC monotherapy in patients with mild to moderate AD showed improvement of cognition [131], that awaits to be validated in a forthcoming large scale phase 3 clinical trial [131].

Other potential therapeutic approaches

A causal link between an impairment of nerve growth factor (NGF) pathway, activation of the amyloidogenic pathway and neurodegeneration in the AD brain has been proposed [132]. Targeted delivery of NGF to basal forebrain cholinergic neurons improves cognitive function in animal models of AD [132]. However, because protein growth factors do not cross the BBB, strategies targeting neurotrophic factors have been poorly exploited so far. Early studies, based on intracerebroventricular (ICV) infusion of NGF, showed a positive effect on cognitive function but were hampered by severe adverse effects related to ICV administration [133, 134]. To overcome these problems, the implant of autologous fibroblasts, genetically modified to express NGF into selected areas of CNS, has recently been proposed [135]. Other strategies use NGF gene-delivery through viral vectors [136–138] (NCT00876863 and NCT00087789**)**. Encapsulated cell bio-delivery (ECB) provides NGF to cholinergic basal forebrain neurons through the stereotactic implantation of a catheter-like device containing NGF-producing cells (NsG0202). Preliminary results suggest a good safety and tolerability of NsG0202 [139].

Aβ triggers mitochondrial dysfunction through a number of pathways [140]. Rescue of mitochondrial function has been therefore considered as a new target to develop disease modifying drugs [141]. Latrepirdine is a weak inhibitor of cholinesterases and a low-affinity NMDA receptor antagonist, which exerts its neuroprotective effects through the stabilization of mitochondria via inhibition of mitochondrial permeability transition pores induced by Aβ [142]. However, the ability of latrepirdine to improve cognition in AD is controversial, due to a discrepancy between the positive signal reported in a phase 2 clinical trial [143] and the subsequent null effect observed in a phase 3 trial [144]. Two RCTs are ongoing to assess the clinical efficacy of latrepirdine in combination with donepezil and memantine (NCT00829374 and NCT00912288). EGCG, mentioned above as an inhibitor of Aβ aggregation, may also inhibit the release of apoptosis-inducing factor (AIF) from mitochondria [145].

Finally a new pharmacological target proposed for developing neuroprotective drugs in AD is the receptor for advanced glycation endproducts (RAGE), a transmembrane protein that belongs to the immunoglobulin superfamily localized in neurons, microglia, astrocytes and the BBB [146]. RAGE mediates the effects of Aβ on microglia, the BBB and neurons through different signaling pathways. RAGE enhances generation and accumulation of Aβ in the CNS by modulating BACE1 [147] and also promotes the transport of Aβ from vascular circulation to the brain. Data from autopsy brain tissues, *in vitro* cell cultures and transgenic mouse models suggest that the Aβ-RAGE interaction exaggerates neuronal stress, impairs learning memory and induces neuroinflammation [148]. A phase 2 trial with PF04494700, a RAGE antagonist, has been recently completed in mild to moderate AD patients and results on clinical efficacy of this drug are awaited in the next months.

Deep brain stimulation (DBS) of memory circuits has been proposed as an alternative, non-pharmacological approach for AD treatment [149]. A recent phase I trial conducted in six mild AD patients, receiving continuous stimulation for 12 months, suggests that DBS can revert impaired glucose utilization in the temporal and parietal lobes as assessed by PET and also slows cognitive decline. Additional studies are needed to confirm these preliminary results.

Limitations and future directions

The pharmacological treatment of AD actually involves cholinesterase inhibitors and memantine, which provide mainly symptomatic short term benefits without counteracting the progression of the disease. Drug discovery in AD has attempted in the last decade to develop disease

modifying drugs with the help of preclinical models, but none of these drugs has succeeded in phase 3. Factors that might explain this failure include suboptimal study design (lack and/or inadequate biomarkers and outcome measurements) and, most importantly, time course of treatment in relation to the development of disease. Available data from failed phase 3 studies suggest that mild to moderate AD patients may be too late in the disease process to improve substantively their outcome following drug treatment.

New criteria for the diagnosis of AD have enlarged the window for the detection of the early stages of the disease and include biomarkers mechanistically related to AD pathology. Adoption of these early biomarkers in implementing design of future studies is highly desirable. Finally, the heterogeneity of AD should be considered in the future when planning RCTs to evaluate the efficacy of disease modifying drugs. Because AD is heterogeneous in terms of clinical presentation, diagnostic issues, underlying neuropathology and mixed causes of dementia have been described in many late onset AD patients, a major challenge will be to identify subgroups with homogeneous biomarkers and to improve the neuropsychological tools for detecting deficits of episodic memory in amnestic MCI patients at high risk to convert into AD. At present, the focus in AD drug development is shifting from treatment to prevention [150]. The new strategy will examine the potential neuroprotective activity of disease modifying drugs in the presymptomatic stages of AD, with the help of biomarkers that predict disease progression before development of overt dementia.

Competing Interests

There are no competing interests to declare.

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